American Journal of Pathology, Vol. 152, No. 2, February 1998


# Proteoglycan Distribution in Lesions of Atherosclerosis Depends on Lesion Severity, Structural Characteristics, and the Proximity of Platelet-Derived Growth Factor and Transforming Growth Factor-β

Stephen P. Evanko,* Elaine W. Raines,* Russell Ross,* Leslie I. Gold,† and Thomas N. Wight*

*From the Department of Pathology,* University of Washington, Seattle, Washington, and the Department of Pathology,† New York University Medical Center, New York, New York*

**The accumulation of proteoglycans (PGs) in atherosclerosis contributes to disease progression and stenosis and may partly depend on local regulation by growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β. In this study, the distribution of the major extracellular PGs is compared with that of PDGF and TGF-β isoforms in developing lesions of atherosclerosis from hypercholesterolemic nonhuman primates. Strong immunostaining for decorin, biglycan, versican, and hyaluronan is observed in both intermediate and advanced lesions. Perlecan staining is weak in intermediate lesions but strong in advanced lesions in areas bordering the plaque core. Immunostaining for PDGF-B and TGF-β1 is particularly prominent in macrophages in intermediate and advanced lesions. In contrast, TGF-β2 and TGF-β3 and PDGF-A are present in both macrophages and smooth muscle cells. Overall, PG deposits parallel areas of intense growth factor immunostaining, with trends in relative localization that suggest interrelationships among certain PGs and growth factors. Notably, decorin and TGF-β1 are distributed similarly, predominantly in the macrophage-rich core, whereas biglycan is prominent in the smooth muscle cell matrix adjoining TGF-β1-positive macrophages. Versican and hyaluronan are enriched in the extracellular matrix adjacent to both PDGF- and TGF-β1-positive cells. These data demonstrate that PG accumulation varies with lesion severity, structural characteristics, and the proximity of PDGF and TGF-β. *(Am J Pathol 1998, 152:533–546)***

Accumulation of extracellular matrix in the artery wall contributes significantly to lesion mass in atherosclerosis and in restenosis after angioplasty.[1–6] Proteoglycans (PGs) and hyaluronan are major nonfibrillar components of the extracellular matrix that have the potential to affect lesion development by regulating events such as lipid accumulation, thrombosis, and cell proliferation and migration and by affecting the material properties of the tissue (reviewed in Ref. 1). At least three types of PGs, as defined by their glycosaminoglycan content and core protein sequence, are present in the extracellular matrix of the artery wall and are synthesized by vascular cells *in vitro*.[1] These include a large aggregating chondroitin sulfate PG, versican,[7] which interacts with hyaluronan to form large multimolecular aggregates; small leucine-rich dermatan sulfate PGs, such as decorin and biglycan, which interact with fibrillar matrix components like collagen and elastin;[8–10] and heparan sulfate PGs, such as perlecan, which is a component of the basal lamina.

PG accumulation in vascular disease may be regulated by locally released growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β.[3,11,12] Stimulation of cultured smooth muscle cells (SMCs) by PDGF leads to an increase in hyaluronan synthesis[13] and up-regulation of versican synthesis and post-translational modifications of the glycosaminoglycan chains,[11] which may promote increased lipid retention.[14,15] TGF-β1 has been shown to be five times more effective than PDGF, epidermal growth factor, and fibroblast growth factor in stimulation of smooth muscle PG synthesis *in vitro*,[16] but it is unclear whether other isoforms of TGF-β are similarly effective. Distinct temporal and spatial patterns of expression of

Supported in part by a grant from the National Heart, Lung, and Blood Institute of the National Institutes of Health (HL-18645 to R. Ross, E. W. Raines, and T. N. Wight and RR-00166 to the Primate Center, University of Washington) and a grant from Bayer AG. S. P. Evanko was supported by a fellowship from the American Heart Association, Washington.

Accepted for publication November 6, 1997.

Address reprint requests to Dr. Thomas N. Wight, Department of Pathology, Box 357470, University of Washington School of Medicine, Seattle, WA 98195. E-mail: tnw@u.washington.edu.

TGF-β1, TGF-β2, and TGF-β3 have been demonstrated during excisional wound repair *in vivo*[17,18] and pulmonary fibrosis,[19] but information on the relative localization of these isoforms in arterial disease is limited. Versican and biglycan expression are up-regulated by TGF-β1 in SMCs with minimal effects on decorin expression.[11,12] Thus, a particular pattern of PGs may reflect specific regulation by locally released growth factors.

Although there are limited data regarding growth factor and PG distribution in injury models[2–5,20] and atherosclerosis,[21–23] the relative distribution of PGs that accumulate in developing lesions together with the factors that may locally regulate PG deposition have not been evaluated. Therefore, we have asked whether a particular pattern of PG accumulation is observed in developing primary lesions of atherosclerosis in hypercholesterolemic nonhuman primates and how PG accumulation relates to the localization of two potent stimulators of PG synthesis, PDGF and TGF-β. We demonstrate that the type of PG deposited in lesions of atherosclerosis depends on lesion severity, structural features of the lesion, and coincident or adjacent localization of PDGF and TGF-β.

## Materials and Methods

### Tissue

Segments of thoracic or abdominal aorta from the nonhuman primate *Macaca nemestrina* were fixed with methyl Carnoy's and embedded in paraffin. Serial sections (~6 μm thick) were cut and placed on Superfrost/plus slides (Fisher Scientific, Pittsburgh, PA). All of the male *M. nemestrina* had been hypercholesterolemic (0.5% cholesterol diet) for 11 months and were placed on the diet when they were between 3 and 5 years of age.[6] Blood cholesterol levels ranged between 650 and 760 mg/dl at the time of sacrifice. Tissue was chosen to give representative examples of atherosclerosis at varying stages of development, ie, fatty streak to advanced fibrous plaques. The cephalad progression of lesions in the nonhuman primate[6] allows the full spectrum of lesion stages to be obtained from an animal after 6 to 12 months on the diet. Advanced fibrous plaques are present in the iliac arteries and lower abdominal aorta whereas early fatty streaks and intermediate lesions are found in the thoracic aorta. Nine lesions from six cholesterol-fed animals and five normal aortas from animals on a regular diet were examined immunohistochemically as described below.

### Antibodies to PGs, Hyaluronan, and Growth Factors

Rabbit polyclonal antisera specific for the core proteins (amino-terminal peptides) of human biglycan (LF-51) and decorin (LF-30) were generously provided by Larry Fisher, National Institute of Dental Research, Bethesda, MD.[24] LF-51 was used at a 1/500 dilution. IgG from LF-30 was purified using protein A Sepharose (Pharmacia, Piscataway, NJ) and was used at 10 μg/ml. Rabbit antibody specific for the poly E region of human versican, VC-E, was kindly provided by Richard LeBaron (University of Texas at San Antonio, San Antonio, TX) and was used at a 1/500 dilution.[25] A rat monoclonal antibody specific for murine EHS heparan sulfate PG (perlecan) that recognizes the perlecan core protein of human, bovine, and mouse and does not cross-react with basement membrane proteins or fibronectin was purchased from Upstate Biotechnology (Lake Placid, NY) and used at 1 μg/ml. Monoclonal antibodies for PDGF were: PGF007 from Mochida Pharmaceutical Co. (Tokyo, Japan), specific for human PDGF-B (residues 73 to 97 of the mature B-chain), used at 0.8 μg/ml[22]; 6C-51, specific for the long form of PDGF A-chain (immunogen was recombinant human PDGF-$A_{LONG}$), from G. Pierce and J. Tarpley (Amgen, Thousand Oaks, CA), used at 2 μg/ml[26]; and a rabbit polyclonal antibody to PDGF A-chain that was generated by immunization with a peptide corresponding to the amino-terminal 30 amino acids of human PDGF-A (Santa Cruz Biotechnology, Santa Cruz, CA). Affinity-purified anti-peptide (19 to 21 amino acids) antibodies specific for each of the TGF-β isoforms 1, 2, and 3[27] and antibodies specific for the corresponding latency-associated peptide (LAP), ie, TGF-β1-LAP, TGF-β2-LAP, and TGF-β3-LAP,[21] were used at 1.25 μg/ml except for the β1-LAP and β3-LAP antibodies, which were used at 2.5 μg/ml. Biotinylated hyaluronan-binding region (bHABR) of aggrecan was used as a specific probe for detection of hyaluronan (4 μg/ml)[28] and was kindly provided by Charles Underhill (Department of Anatomy and Cell Biology, Georgetown University, Washington, DC). Monoclonal antibody HAM 56 to human macrophages[29] and an antibody specific for smooth muscle α-actin generated by immunization with the amino-terminal decapeptide of human smooth muscle α-actin (clone asm-1, Boehringer-Mannheim, Indianapolis, IN) were used to identify macrophages and SMCs, respectively.

### Immunohistochemistry

Sections of aortic specimens were deparaffinized in Histoclear (National Diagnostics, Atlanta, GA) and rehydrated in a descending ethanol series. Slides were incubated in phosphate-buffered saline (PBS) containing 0.3% $H_2O_2$, 0.1% sodium azide for 10 minutes to quench endogenous peroxidase activity. After rinsing in PBS, specimens to be stained with PG antibodies (except perlecan) were digested with chondroitin ABC lyase (ICN Biomedicals, Costa Mesa, CA) at 250 mU/ml in 0.1 mol/L Tris/acetate, pH 7.3, for 1 hour at 37°C. Specimens to be stained with perlecan, TGF-β, and TGF-β-LAP antibodies were digested with *Streptomyces* hyaluronidase (Sigma Chemical Co., St. Louis, MO) at 10 U/ml in 0.1 mol/L sodium acetate, 0.85% NaCl, pH 5.5. The appropriate dilution (see above) of primary antibody was applied in PBS containing 1% bovine serum albumin, and sections were incubated overnight at 4°C (growth factor antibodies, α-actin, and HAM 56) or for 1 hour at room temperature (PG antibodies). After rinses with PBS, a 1/1000 dilution of the appropriate biotin-conjugated secondary antibody (Zymed, South San Francisco, CA) was applied

**Table 1.** Proteooglycan and Hyaluronan Distribution in Experimental Lesions of Atherosclerosis as Compared with Normal Aortas

| | Normal vessel | | Fibrous plaque | | | | |
|---|---|---|---|---|---|---|---|
| PG or GAG | Endothelial cells | SMCs | Endothelial cells | SMCs | Macrophages | Fibrous cap | Plaque core |
| Perlecan | +++ | ++ | +++ | +++ | ++ | + | +++ |
| Decorin* | + | ++ | + | + | +++ | + | +++ |
| Biglycan | −/+ | ++ | ++ | +++ | + | +++ | −/+ |
| Versican | − | ++ | −/+ | +++ | − | +++ | − |
| Hyaluronan | ++ | + | ++ | +++ | +++ | +++ | +++ |

Results are expressed as follows: −, undetectable staining; −/+, variably detectable; +, detectable staining; ++, moderate staining; +++, strong staining. *Staining for PGs was primarily extracellular, except for decorin, which also showed intracellular staining. Column heads indicate the major cell type or lesion component in the vicinity of intense staining. Note that these data reflect the overall relative staining intensity within lesions and do not necessarily reflect actual quantitative differences between different PGs. Reliable comparisons can be made only between cell types or between structural features.

and incubated for 30 minutes at room temperature. Slides were washed three times and then incubated with a 1/5000 dilution of streptavidin conjugated to horseradish peroxidase for 10 minutes. Color was developed with diaminobenzidine, and sections were counterstained with methyl green. Contrast of the brown reaction product was enhanced using a BG12 filter during photomicrography, and all micrographs were printed under identical conditions. Control sections were incubated with the proper dilution of normal serum or IgG to match the conditions or isotype of the specific antibodies. Specificity of hyaluronan staining was verified by abolition of staining by pretreatment of the sections with *Streptomyces* hyaluronidase (data not shown). The specificity of the PDGF antibodies was demonstrated by abolition of staining with a 10-fold molar excess of the appropriate PDGF isoform[23,27] (data not shown).

## Results

### Proteoglycan Distribution Depends on Lesion Stage and Structural Features

Normal nonhuman primate arteries were compared immunohistochemically with those after diet-induced hypercholesterolemia to evaluate changes in the distribution of the PGs perlecan, decorin, biglycan, and versican and the glycosaminoglycan hyaluronan. In the normal aortic media (Table 1), immunostaining for the four PGs and hyaluronan is relatively uniform in the matrix surrounding the SMCs, with occasional patches of increased immunoreactivity. Staining for perlecan and hyaluronan is prominent in the endothelial basement membrane, whereas decorin, biglycan, and versican staining is faint directly around the endothelium but more intense in the deeper, loose matrix underlying the endothelium (data not shown). Biglycan immunostaining is closely associated with elastic fibers. In the adventitia, prominent staining of decorin and hyaluronan is observed, but no immunostaining is detected with antibodies to versican, biglycan, or perlecan.

To evaluate changes associated with developing lesions of atherosclerosis, lesions from animals fed a high-cholesterol diet for 11 months were examined. The plasma cholesterol levels in these animals (650 to 760 mg/dl) are very high and are comparable to those observed in patients with type II familial hypercholesterolemia. However, the cellular changes observed in animals with very high-level hypercholesterolemia are similar to those observed in animals with low-level hypercholesterolemia whose plasma cholesterol levels (200 to 400 mg/dl) more closely mimic individuals at risk for atherosclerosis in the general population.[30,31] With low-level hypercholesterolemia, the cellular changes progress more slowly. The cephalad progression of atherosclerotic lesions in the nonhuman primate means that animals sacrificed after 6 to 12 months of diet administration will have a spectrum of lesions throughout their aortic tree. Depending on the level of hypercholesterolemia, an animal can have extensive fibrous plaques in the iliac arteries and lower abdominal aorta, whereas early fatty streaks and mixed intermediate lesions will be found in the thoracic aorta.

In early fatty streaks, PG immunostaining is generally weak around the thin, subendothelial layer of foamy macrophages but is stronger and seen in a patchy distribution in the underlying media (data not shown). Perlecan immunostaining is strong in the subendothelial basement membrane as seen in the normal vessels. In intermediate lesions, characterized by the presence of both SMCs (Figure 1A) and macrophages (Figure 1B), there is a differential distribution of the four PGs and hyaluronan. Perlecan immunostaining (Figure 1D) is generally more intense in the media than in the developing neointima. In contrast, antibodies to decorin (Figure 1E) stain the neointima somewhat more intensely than the media, and staining is strongest in the luminal region (inner third) of the neointima in the developing fibrous cap. The antibody for biglycan (Figure 1F) prominently stains the neointima of early-intermediate lesions. The pattern of biglycan immunostaining appears to be associated with the SMC-rich regions of the lesions and closely matches the pattern of $\alpha$-actin immunostaining (compare Figure 1, A and F). There are some areas of $\alpha$-actin positivity, such as directly above the internal elastic lamina, with less apparent staining for biglycan or the other PGs. This is mainly due to the longitudinal orientation (and thus transverse sectioning) of these SMCs, which renders the extracellular matrix between them less visible. Intermediate lesions stain intensely with the versican antibody and the hyaluronan-binding protein as compared with the underlying media. Versican immunostaining (Figure 1G) is associated primarily with the matrix in SMC-rich areas,

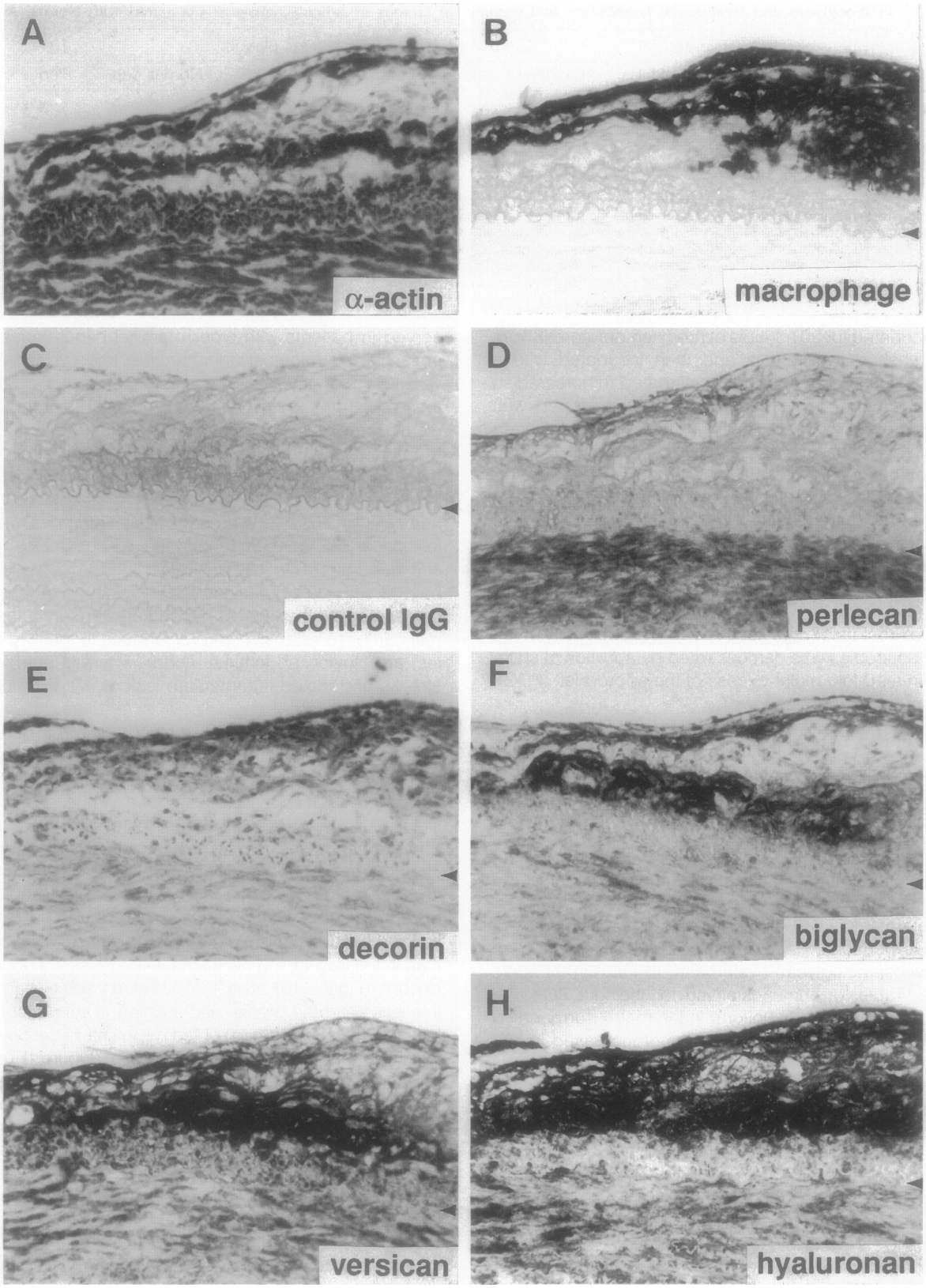


**Figure 1.** Increased levels of some PGs and the growth factors PDGF and TGF-β in intermediate lesions. Sections of abdominal aorta were immunostained with antibodies specific for the molecules indicated on each micrograph. Antibodies to biglycan (**F**) and versican (**G**) and a biotinylated probe for hyaluronan (**H**) show strong neointimal staining compared with underlying media and normal vessels (data not shown). Also note the similarity in localization of decorin (**E**) and TGF-β1 (**I**) immunostaining and of staining for α-actin (**A**), biglycan (**F**), and the long form of PDGF A-chain (**J**). The internal elastic lamina (IEL) is indicated with an **arrowhead**. Micrographs were photographed and printed under identical contrast conditions. Magnification, ×150.

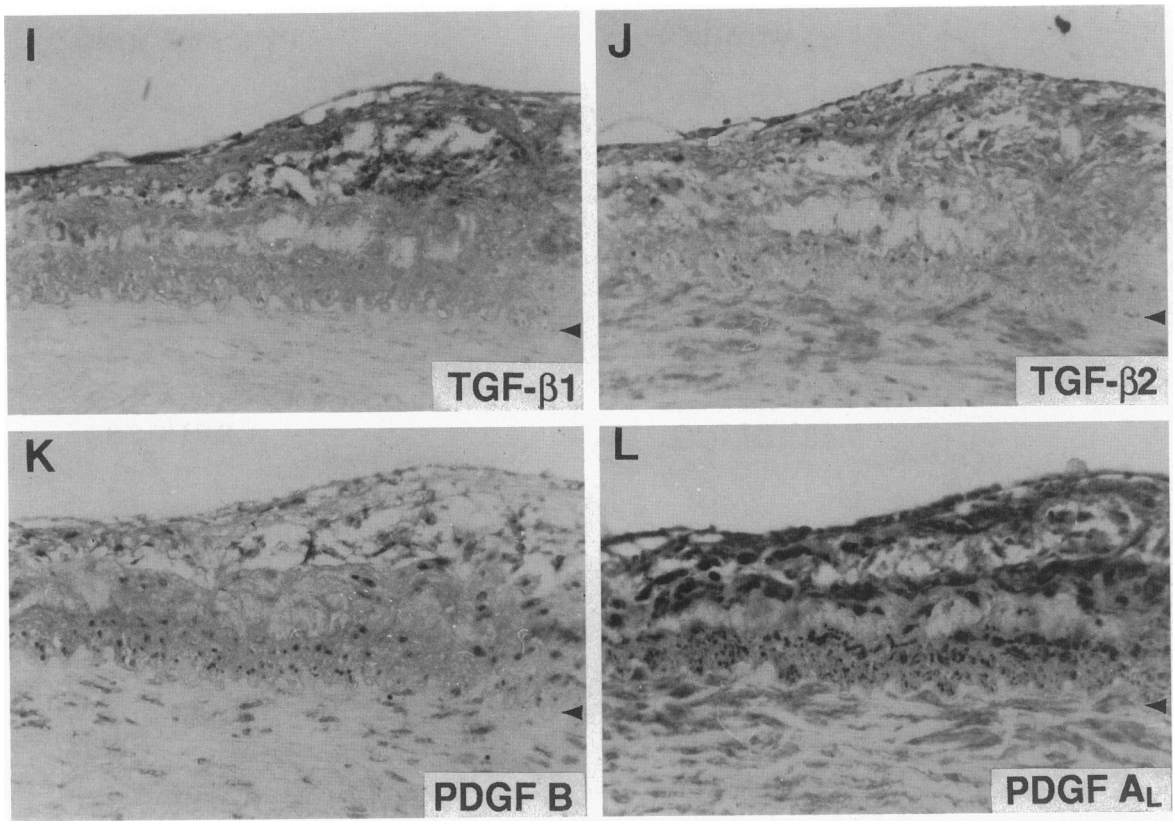


**Figure 1.** (Continued)

whereas hyaluronan (Figure 1H) is detected throughout the lesions around both SMCs and macrophages. In the media beneath the intermediate lesions, PG staining is similar to that of the media in normal tissue, ie, diffuse staining with patches of strong immunoreactivity. Therefore, in this and similar intermediate lesions, neointimal staining for versican, hyaluronan, biglycan, and decorin is stronger than the underlying media, whereas perlecan immunostaining in the lesion is weaker than the underlying media. Staining for biglycan, versican, and hyaluronan is particularly strong where SMCs and macrophages are closely apposed.

In more advanced lesions (Figure 2 and Table 1), localization of specific PGs appears to correlate with particular structural features of the plaque. For example, perlecan immunostaining (Figure 2D) is particularly enriched around the margin of the plaque core where there is a mixture of SMCs and macrophages. Here, perlecan is seen in thin structures that closely match the distribution of $\alpha$-actin-positive cells. Perlecan is also observed in the superficial cap and endothelial basement membrane, and patches of stronger perlecan immunostaining are observed in the inner media directly beneath the lesions. Occasionally, perlecan staining is virtually absent in some areas of the media (see arrowhead in Figure 2D). Another consistent finding is the enrichment of versican immunostaining in the fibrous cap and plaque margins (Figure 2G) but an absence of staining in the macrophage-rich plaque core. In direct contrast, staining for hyaluronan (Figure 2H) is prominent in both the fibrous cap and plaque core.

Decorin (Figure 2E) and biglycan (Figure 2F) immunostaining in more advanced lesions is partly overlapping but mostly discordant. For example, when Figure 2, E and F, are compared, immunostaining for both biglycan and decorin is localized to the the fibrous cap. However, decorin immunostaining is strong in macrophage-rich regions, hugging the plaque core and in the superficialmost layer of the fibrous cap. In contrast, biglycan immunostaining is more widely distributed around SMCs of the intima and is less intense in the atheromatous core. There were also overlapping and exclusive areas of biglycan and perlecan immunostaining (compare Figure 2, D and F). Furthermore, biglycan staining is consistently observed in close association with elastin, both in the internal elastic lamina and in the (presumably) newly forming elastin sheets in the neointima (Figure 3A). This is found in normal and many diseased specimens and is not necessarily related to lesion stage. On the other hand, neither decorin (Figure 3B), which was previously localized to elastic fibers immunohistochemically in human dermis,[10] nor perlecan (see Figure 2) are observed in close association with elastin in aortic tissue.

## *PDGF and TGF-β Isoform Expression Varies with Cell Type*

The regional variation in PG staining intensity within lesions suggests that there are local differences in PG deposition and/or degradation. As both PDGF and TGF-β

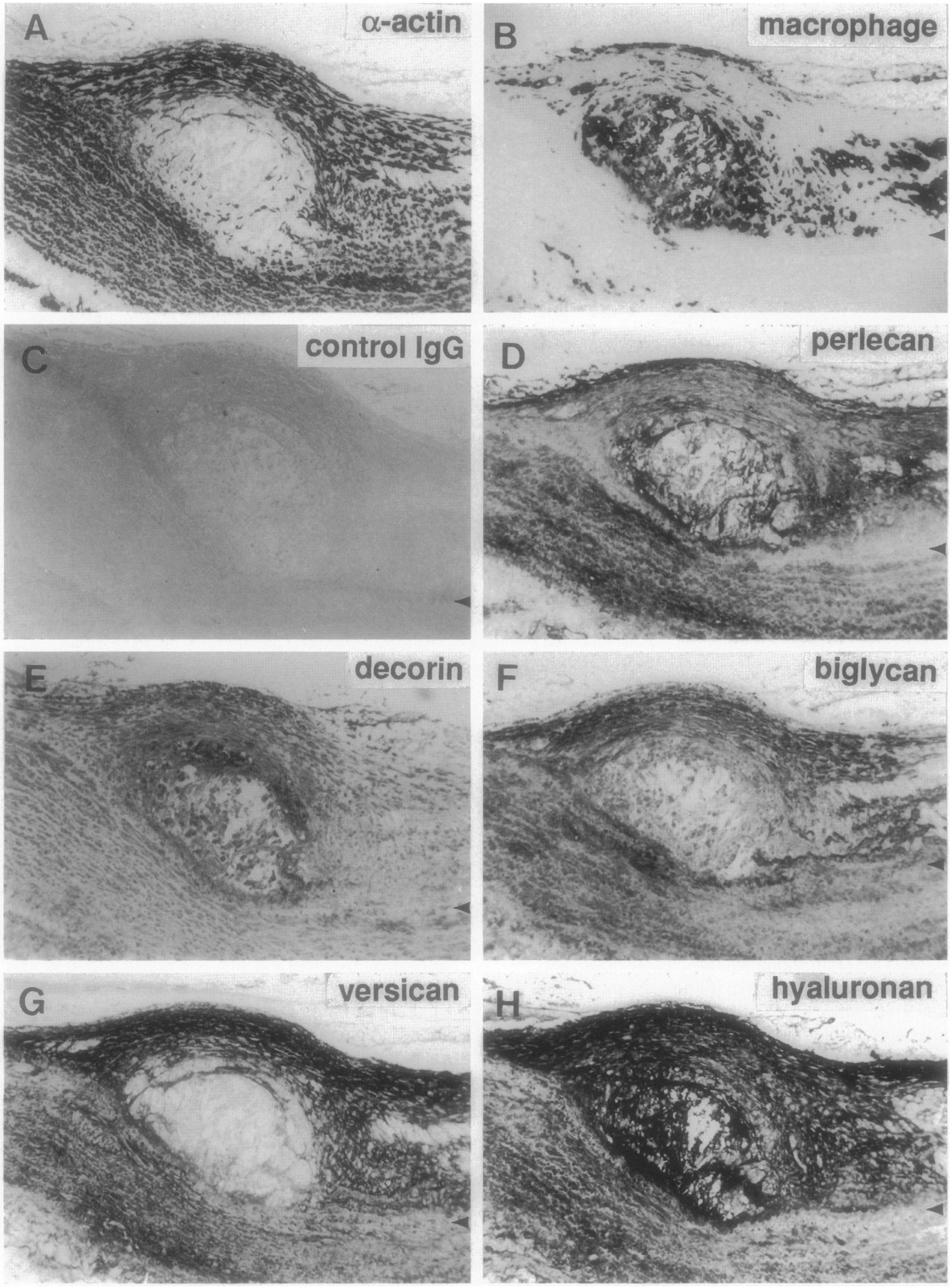


**Figure 2.** PGs, PDGF, and TGF-β have distinct distributions in advanced fibrous plaques. Intense staining with antibody to perlecan (**D**) is observed in the SMCs bordering the plaque core, in the subendothelial matrix, and in patches in the underlying media. Biglycan (**E**) and decorin (**F**) exhibit overlapping and exclusive immunostaining in the fibrous cap and plaque core. Versican immunostaining (**G**:) is strong in the fibrous cap but is not seen in the plaque core, whereas hyaluronan (**H**) is localized to both regions. TGF-β and PDGF staining (I to L) is strongest in the macrophage-rich plaque core, but TGF-β2 and PDGF A staining is also detected in plaque and medial SMCs. Note similarity between decorin (**E**) and TGF-β1 (**I**) and -β2 (**J**) immunostaining. The **arrowhead** indicates the position of the IEL. Magnification, ×60.

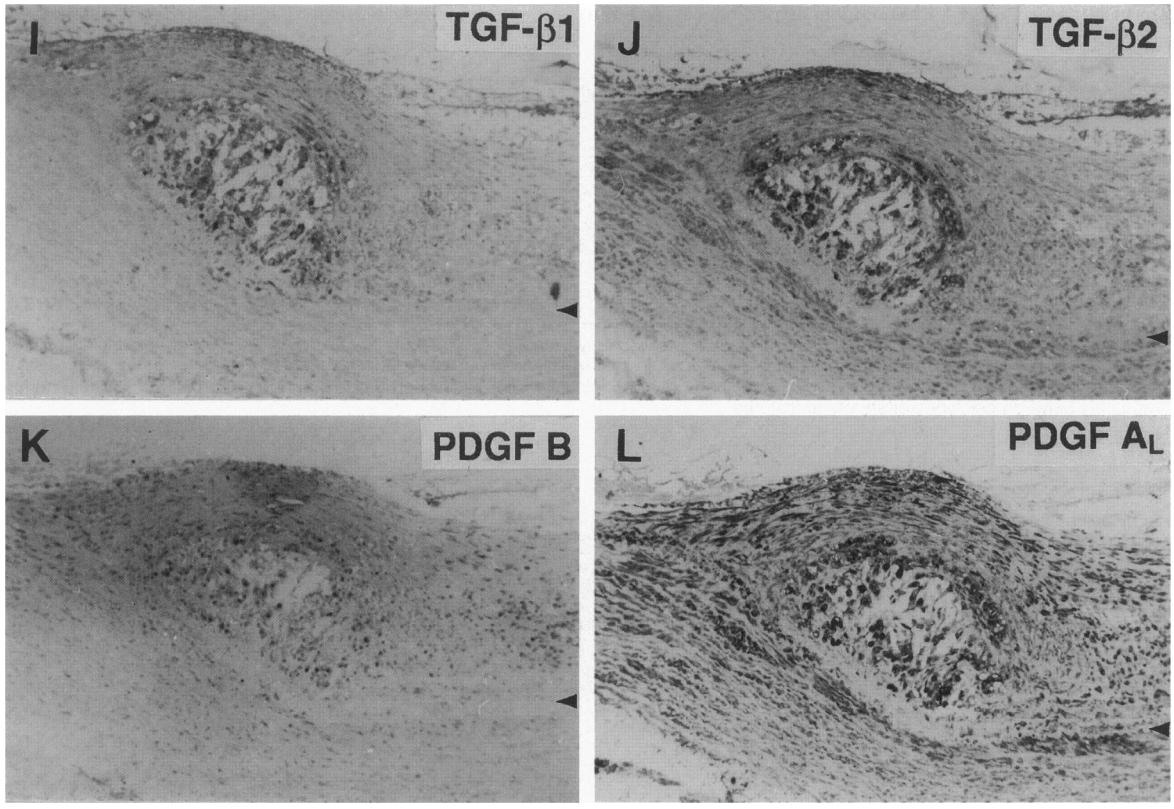


**Figure 2.** (Continued)

are known to stimulate specific PG synthesis by vascular cells,[11,12] adjacent sections were stained with antibodies to different isoforms of these growth factors. General results regarding cell type and topographical distribution of immunoreactivity for these growth factors are summarized in Table 2. In normal vessels, immunostaining for PDGF-B and TGF-$\beta$ is generally weak or absent (Table 2) but is present in macrophages in early fatty streaks (data not shown). However, immunostaining for PDGF-A and TGF-$\beta$2 and -$\beta$3 is detectable in the SMCs of normal vessels and in both macrophages and SMCs in early fatty streaks.

In intermediate and advanced lesions, immunostaining for PDGF B-chain (Figures 1K and 2K) and TGF-$\beta$1 (Figures 1I and 2I) is primarily observed in macrophage-rich areas, with weaker staining of SMCs. Occasionally, strong PDGF-B immunostaining is observed in the SMCs of the media directly underlying a developing lesion (Figure 1K). In contrast, PDGF A-chain (Figures 1L and 2L) is clearly associated with intimal SMCs, based on co-localization with $\alpha$-actin-positive cells, but is also seen in macrophage-rich zones and directly adjacent to the lumen. This same immunostaining pattern is observed using two separate antibodies to PDGF-A, one specific for

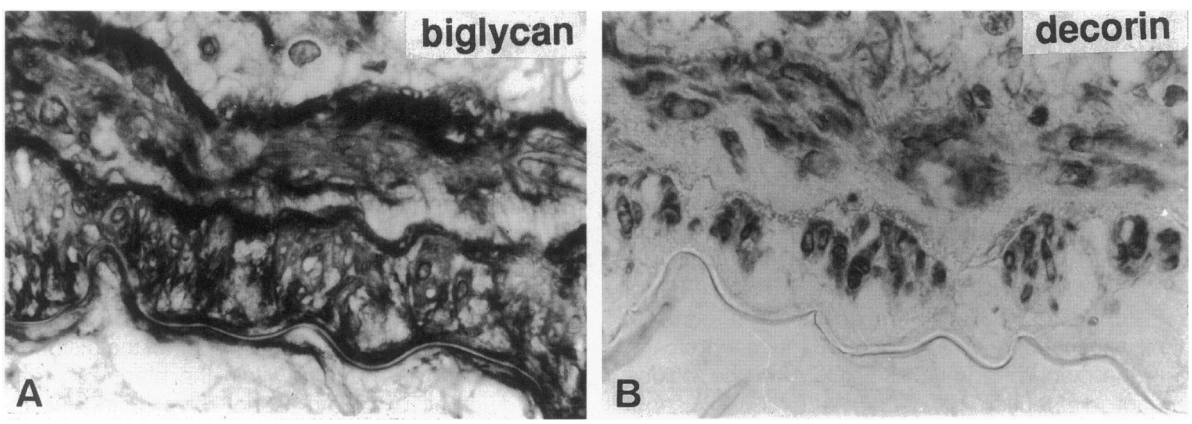


**Figure 3.** Biglycan is concentrated in areas of elastic fibers. High-power micrograph from the base of an intermediate lesion in which the IEL and newly forming elastic laminae in the neointima are strongly positive for biglycan (**A**) but not decorin (**B**) immunostaining. Magnification, ×580.

**Table 2.** PDGF and TGF-β Localization in Experimental Lesions of Atherosclerosis as Compared with Normal Vessels

| | Normal vessel | | Fibrous plaque | | | | |
|---|---|---|---|---|---|---|---|
| Growth factor | Endothelial cells | SMCs | Endothelial cells | SMCs | Macrophages | Fibrous cap | Plaque core |
| PDGF B | − | −/+ | − | −/+ | +++ | −/+ | +++ |
| PDGF A | + | ++ | −/+ | +++ | ++ | +++ | ++ |
| TGF-β1 | − | − | + | −/+ | +++ | + | +++ |
| TGF-β1 LAP | + | − | ++ | + | +++ | ++ | +++ |
| TGF-β2 | −/+ | −/+ | + | ++ | +++ | ++ | +++ |
| TGF-β2 LAP | + | + | ++ | +++ | +++ | ++ | +++ |
| TGF-β3 | −/+ | −/+ | ++ | ++ | +++ | ++ | +++ |
| TGF-β3 LAP | ++ | ++ | ++ | +++ | +++ | ++ | +++ |

Results are expressed as follows: −, undetectable staining; −/+, variably detectable; +, detectable staining; ++, moderate staining; +++, strong staining. Staining for all growth factors was intracellular. Little or no matrix staining was detected. Note that these data reflect the overall relative staining intensity within lesions and do not necessarily reflect actual quantitative differences between different growth factors. Reliable comparisons can be made only between cell types or structural features.

the long form (ie, containing exon-6-encoded sequence, Figure 1L), and the other, which recognizes both the long and short isoforms (data not shown). Whereas TGF-β1 immunoreactivity is most prominent in macrophages, immunostaining for TGF-β2 (Figure 1J) and TGF-β3 (not shown, see Table 2) is associated with both macrophages and smooth muscle. The regional distributions of TGF-β2 and -β3 are identical. Although there is some diffuse staining of the extracellular matrix in the lesions with antibodies to mature TGF-β and PDGF, there is little evidence for extensive extracellular localization of any growth factor.

TGF-β is secreted as a latent molecule composed of the latency-associated peptide (LAP) and the mature growth factor. On activation by proteases, the mature functional dimer is released.[32] Thus, although antibodies to epitopes on the LAP region or the mature region cannot distinguish active from latent TGF-β, immunostaining with the anti-LAP antibodies most likely indicates the cells that synthesize and secrete the TGF-β proteins. Immunostaining for the LAPs of the respective TGF-β isoforms (TGF-β-LAPs) is similar to that of the mature growth factors except that the immunoreactivity is more intense for the LAPs (Table 2). These data suggest that macrophages, SMCs, and endothelial cells synthesize and secrete all three isoforms of TGF-β.

### *Localization of Specific Proteoglycans Is Related to TGF-β and PDGF Distribution*

When the spatial distribution of staining for PGs and growth factors is compared, patterns emerge that imply possible autocrine and/or paracrine effects on PG accumulation or potential interactions between PGs and growth factors. Although the number of sections between two stained slides varies, structural features of the lesion are similar, and general trends in relative distributions are observed. For example, in the intermediate lesion (Figure 1), the distribution of TGF-β1 immunostaining (Figure 1I) coincides with that of decorin (Figure 1E), in that both are strong in the macrophage-rich inner third of the lesion. Concordant distribution of decorin and TGF-β1 and TGF-β2 is readily apparent in several advanced lesions, particularly in macrophage-rich zones in the plaque core and near the luminal surface (compare Figure 2, E with I and J). Another representative example is shown in Figure 4 in which the immunostaining for TGF-β1 (Figure 4D) and decorin (Figure 4E) is intense in the macrophages at the luminal surface and in the plaque core of an intermediate-advanced lesion. In contrast, biglycan (Figure 4F) is deposited in large amounts in the adjacent intervening SMC matrix, particularly beneath the TGF-β1-positive macrophages at the luminal surface. It should also be noted that, in this example, the macrophages at the luminal surface, which are positive for TGF-β1, are negative for PDGF-B (Figure 4B) and PDGF-$A_L$ (Figure 4C), indicating variability in growth factor expression by different populations of macrophages.

Evidence that PG deposition may reflect paracrine stimulation by growth factors is provided in Figure 5. Biglycan immunostaining (Figure 5B) is especially abundant in the smooth muscle matrix in areas immediately adjacent to macrophages, which are strongly positive for TGF-β1 (Figure 5A, upper part of the lesion), but less intense adjacent to cells positive for PDGF B-chain (Figure 5C, lower part of the lesion). In contrast, versican immunoreactivity (Figure 5D) is present in the smooth muscle matrix adjacent to both TGF-β1- and PDGF-B-positive macrophages. Figure 6 illustrates at higher magnification the consistently observed close proximity of intense biglycan staining (Figure 6, B and D) adjacent to TGF-β-positive macrophages (Figure 6, A and C), perhaps reflecting stimulation of biglycan in SMCs by locally released TGF-β.

## *Discussion*

### *Proteoglycan Involvement in Lesion Structure and Matrix Remodeling*

This study has demonstrated that in lesions of atherosclerosis in nonhuman primates the accumulation of specific PGs varies with lesion severity and with the distribution of cells and growth factors within the plaque, suggesting that different PGs play distinct roles during lesion progression. Altered levels of specific PGs may directly affect material properties of the tissue via their contribution

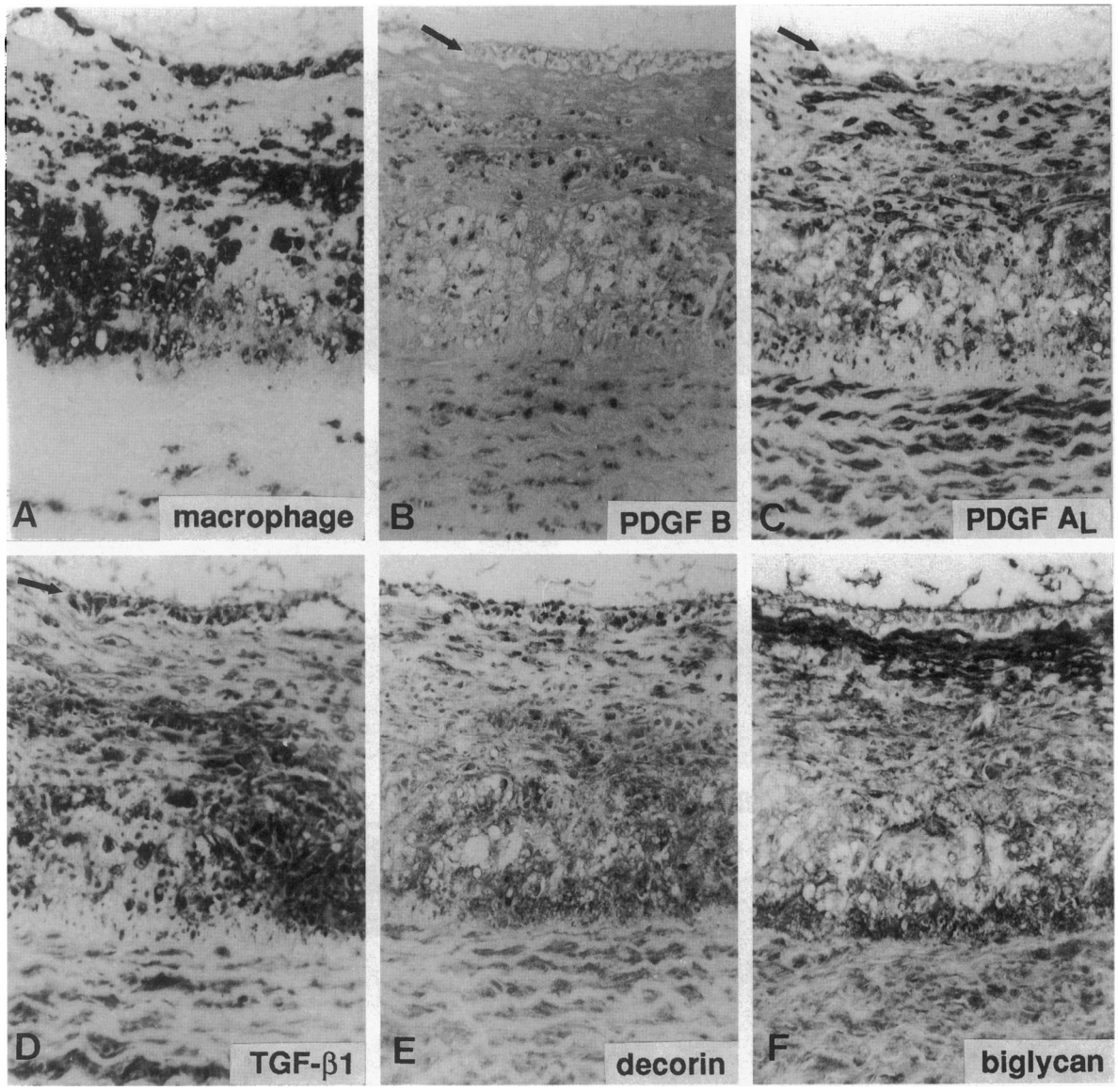


**Figure 4.** Biglycan and decorin have distinct and overlapping distributions. Note the similarity of TGF-β1 (**D**) and decorin immunostaining (**E**), which is associated mainly with macrophage-rich areas. In contrast, biglycan immunostaining (**F**) is enhanced in the SMC matrix in the fibrous cap between decorin and TGF-β-positive areas, and also just above the IEL. The variability of TGF-β and PDGF expression by macrophages within the lesion is also illustrated. Note the lack of staining for PDGF B-chain (**B**) and PDGF $A_L$ (**C**) but strong staining for TGF-β1 (**D**) in the more luminal macrophages (**arrows**). Magnification, ×150.

to swelling or indirectly by their potential to affect polymerization, degree of packing, and structural arrangement of fibrous matrix components such as collagen or elastin. Ultrastructural studies have shown that large chondroitin sulfate PGs (ie, versican) are found in pockets between bundles of collagen fibrils, and dermatan sulfate PGs (ie, decorin) are within collagen fibril bundles.[23] Targeted disruption of decorin expression leads to abnormal collagen fibril morphology and skin fragility.[33] Therefore, the presence of increased levels or specific combinations of PGs, such as decorin or versican and hyaluronan in the fibrous cap and plaque margins, can influence the mechanical continuity or isotropy of the fiber system[34] and thus directly affect the susceptibility of the plaque to rupture.

A number of studies have localized chondroitin sulfate PGs in the matrix of normal and atherosclerotic arteries.[23,35–38] However, it has only recently become clear that the bulk of the chondroitin sulfate in vessels is associated with versican.[2,7] Versican is known to bind hyaluronan, and together they form large multimolecular aggregates.[39] Our observation that versican and hyaluronan show similar localization in the SMC matrix suggests that aggregate formation may occur in lesions of atherosclerosis. The marked increase in versican and hyaluronan in early lesions also suggests that they may play a role in the early events of atherogenesis, such as proliferation and migration of SMCs and leukocytes. Hyaluronan and versican synthesis is up-regulated in proliferating cells after stimulation with serum or PDGF.[11,13]

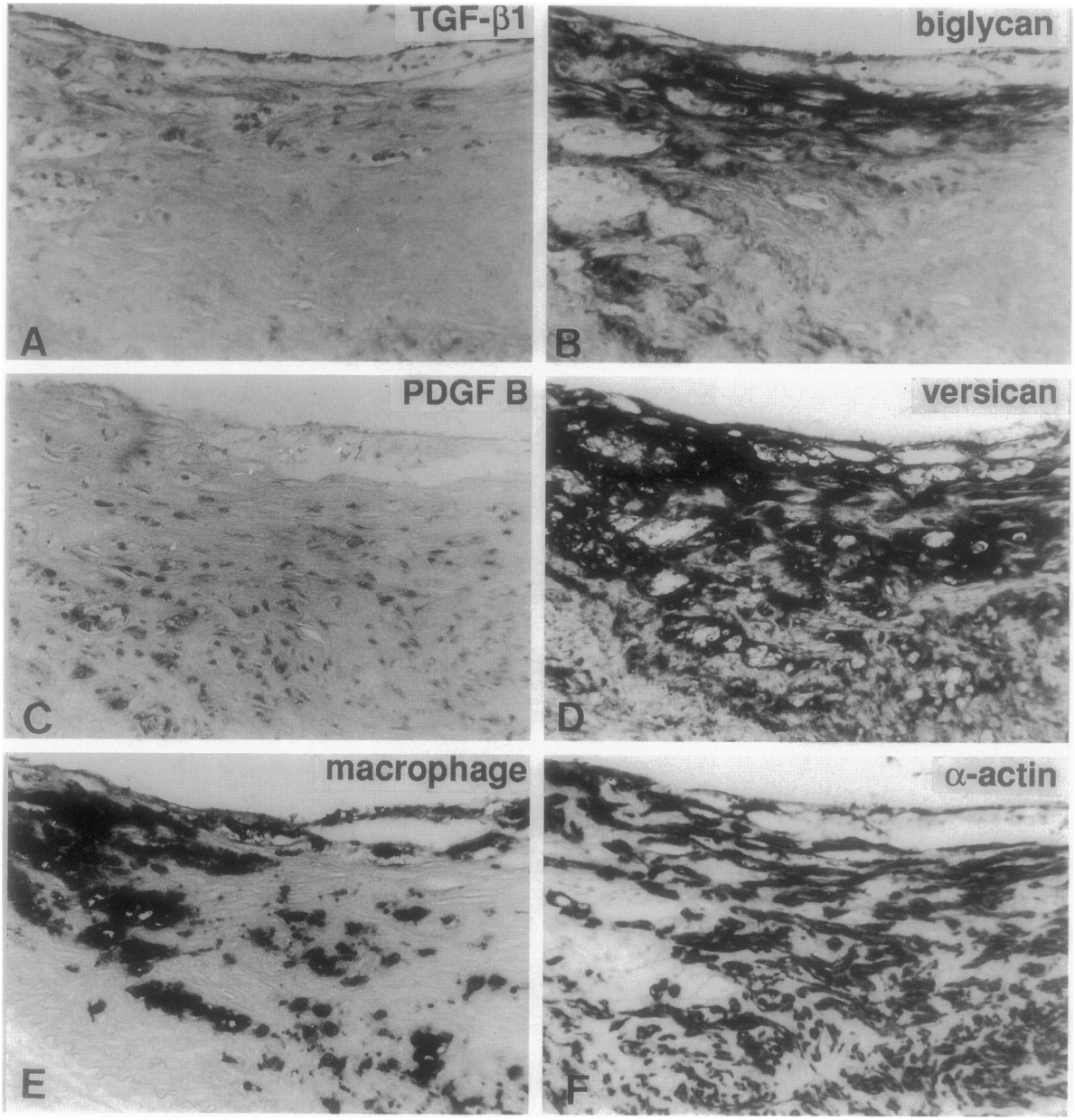


**Figure 5.** PG staining patterns suggest the paracrine action of growth factors on PG accumulation. Biglycan (**B**) is deposited in the SMC matrix adjacent to TGF-β1-positive macrophages in the upper part of the neointima. Versican (**D**) accumulations are seen in the SMC matrix around both TGF-β- and PDGF-positive macrophages. The IEL can be seen in the lower left corner of the field. Magnification, ×150.

Furthermore, hyaluronan and versican are principal matrix components of human restenotic lesions[5,40] and have been shown to contribute to neointimal thickening after vascular injury *in vivo*[3,5] and play a role in locomotion and mitosis of cells *in vitro*.[41,42] Organization of hyaluronan and versican-rich pericellular matrices may modulate cell attachment to fibrillar matrix components, thereby facilitating detachment necessary for locomotion or mitosis[42] (Evanko and Wight, unpublished observations). An abundance of versican early in atherogenesis could also predispose the extracellular matrix to increased lipid entrapment due to the binding of lipoproteins by the chondroitin sulfate chains,[14,15] an idea supported by co-localization of versican with apolipoprotein (a) and apolipoprotein E in transplant arteriopathy.[43]

Recent studies indicate that versican is a substrate for metalloproteinases such as stromelysin, matrilysin, and gelatinase.[44,45] The absence of versican immunostaining in the plaque core in advanced lesions may be the result of degradation by these macrophage-derived proteases[44,46] as matrilysin was shown to be confined to the border between the lipid core and the overlying fibrous areas in advanced human lesions.[44] Loss of versican from the plaque may result in instability of the matrix in a manner similar to that seen when the chondroitin sulfate PG aggrecan is lost from cartilage in osteoarthritis.[47]

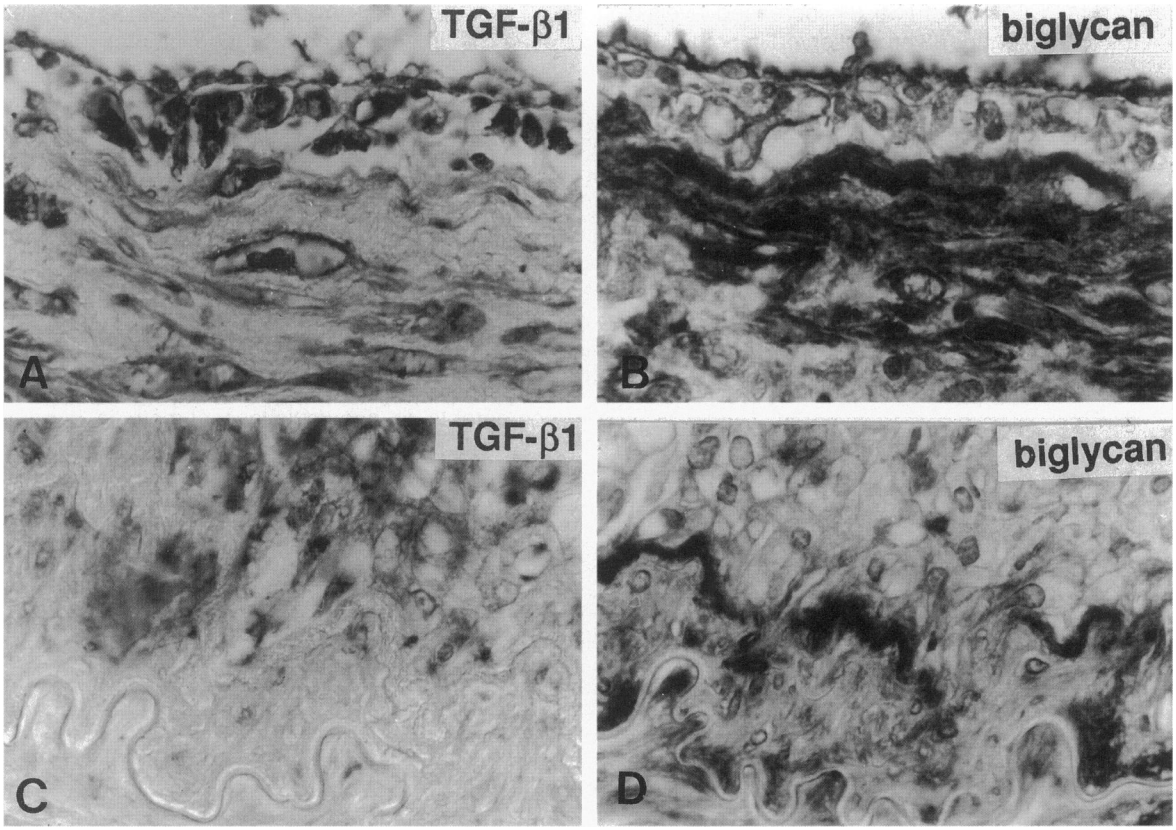


**Figure 6.** Biglycan deposition parallels TGF-$\beta$ staining of macrophages. High-power micrographs illustrate the close proximity of biglycan accumulation in the matrix of SMC-rich areas that are frequently observed adjacent to TGF-$\beta$1-positive macrophages. TGF-$\beta$1 (**A**) and biglycan (**B**) are adjacently localized near the luminal surface of the same advanced lesion shown in Figure 4 and in a different advanced lesion near the base of the plaque core with TGF-$\beta$-positive macrophages in the core (**C**) and intense biglycan staining (**D**) in the adjacent smooth muscle matrix. Magnification, ×580.

The role of biglycan in arterial cell biology or in the supramolecular organization of the extracellular matrix is unclear. Biglycan and decorin core proteins are highly similar.[24] However, decorin has been shown to bind to collagen and regulate collagen fibril formation,[8,9,33] but there are conflicting reports regarding the binding of biglycan to collagen.[8,9] Recently, reciprocal immunostaining of biglycan and decorin was seen in transplant arteriosclerosis[48] and in human restenotic lesions.[4] In the latter study, biglycan staining of the loose connective tissue was intimately associated with staining for collagen types I and III. Both decorin and biglycan have been localized to elastic fibers in human dermis.[10] Biglycan and tropoelastin mRNA expression was elevated in balloon-injured rat carotid arteries,[2] and our results suggest that biglycan is a major PG associated with elastin in primate arteries and are similar to observations in human coronary arteriopathy.[48] It is possible that this association somehow affects elastin polymerization or cross-linking.

Perlecan is predominantly associated with the media in normal vessels and early stages of lesion formation but is prominent in the SMC matrix near the plaque core of more advanced lesions. Recent studies implicate this basement membrane heparan sulfate PG in regulating autonomous growth by vascular SMCs and suggest that breakdown of the basement membrane and loss of interaction with perlecan releases cells from their quiescent state via regulation of transcription factors.[49] The deposition of perlecan may represent re-establishment of a basement membrane around the SMCs. Therefore, it would be of interest to compare perlecan staining with other basement membrane components, such as collagen type IV, and with markers of SMC phenotype.

## *Locally Released Growth Factors in Developing Lesions of Atherosclerosis: Possible Regulation of Specific Proteoglycans*

Our data demonstrate that the complex mixture of growth factors in developing lesions is dependent on cell type. Neither PDGF-B nor TGF-$\beta$1 are present to any significant extent in normal vessels. Both, however, are particularly prominent in macrophages in intermediate and advanced lesions induced by hypercholesterolemia, consistent with earlier observations made in advanced human lesions.[21,22] Our data also indicate that PDGF production varies with location in the lesion, with examples of more luminal macrophages staining for TGF-$\beta$1 but not for PDGF-B or PDGF-A. This may reflect different macrophage subpopulations or time of entry into the vessel wall or different states of maturation and activation as has previously been reported for other markers of macrophage differentiation within lesions.[50]

In contrast to PDGF-B and TGF-β1, increased levels of PDGF-A and TGF-β2 and -β3 are observed in both SMCs and macrophages in intermediate and advanced lesions. Immunostaining for PDGF-A in the present study is more extensive than previously reported in polytetra fluoro ethylene grafts[51] and in renal vascular rejection.[52] The relatively intense immunostaining for TGF-β2 and -β3 in SMCs, macrophages, and endothelium contrasts somewhat with remodeling pulmonary arteries in which staining for TGF-β was seen primarily in nonfoamy macrophages and endothelial cells.[21] The differential localization of TGF-β1 and TGF-β2 and -β3 in our study is of interest, as separate functional responses to these isoforms have been shown in cultured SMCs and endothelial cells.[53]

The simple presence of isoforms of TGF-β does not conclusively indicate their involvement as activation of the latent TGF-β complex often requires contact between two different cell types and is highly regulated. However, elicited macrophages are able to release active TGF-β1 on their own,[31] and in a model of pulmonary inflammation and fibrosis, alveolar macrophage secretion of active TGF-β1 has been shown to be regulated by coordinate release of plasmin.[54] Thus, although we observed no differences in the immunolocalization of the different mature TGF-β isoforms and their respective latency-associated proteins, the presence of LAP does not mean that all of the TGF-β is inactive. A recent study showed that embryonic mesoderm induction by TGF-β1 occurs only when ligand-producing cells are in direct contact with responsive cells.[55] However, the diffusibility of factors such as TGF-β or PDGF within the vascular extracellular matrix is not known.

The close juxtaposition of PG- and PDGF- and TGF-β1-positive areas suggest that the local combination of growth factors may influence PG accumulation. For example, the concentration of biglycan in SMCs beneath TGF-β1-positive macrophages may reflect paracrine stimulation of biglycan synthesis by macrophage-activated TGF-β1. This would be consistent with studies in the atherosclerotic pulmonary artery in which the active form of TGF-β1 was observed exclusively in macrophages and localized adjacent to type I procollagen mRNA-expressing SMCs.[21] Our observations of intense staining for biglycan adjacent to TGF-β1-positive macrophages, but less intense staining adjacent to PDGF-B-positive, TGF-β1-negative macrophages are consistent with *in vitro* studies that show biglycan expression to be specifically up-regulated by TGF-β1 and not by PDGF in SMCs.[12]

In contrast to biglycan, versican accumulation is observed in SMCs adjacent to both PDGF- and TGF-β1-positive macrophages. Both of these factors increase the synthesis and glycosaminoglycan chain length of cultured SMC-derived versican.[11] Anti-TGF-β1 antibody treatment in the rat carotid artery injury model decreased versican accumulation after injury.[3] Thus, specific PG deposition that parallels PDGF- and/or TGF-β1-positive cells is observed during development of atherosclerosis and is consistent with specific *in vitro* activities of PDGF and TGF-β1 in SMCs.

There is growing evidence that PGs can regulate cell behavior either directly or through interaction with growth factors.[1,49,56,57] For example, both perlecan and decorin have direct growth-suppressive activity.[49,57] In addition, matrix-associated heparan sulfate PGs, such as perlecan, can bind to PDGF $A_{Long}$ and fibroblast growth factor and thus influence their activity.[58,59] Decorin and related PGs have also been shown to interact with TGF-β1 and possibly neutralize its activity.[60] Decorin infusion in a model of glomerulonephritis attenuated extracellular matrix accumulation associated with the disease process, possibly in part through interaction with locally released TGF-β.[61] In our study, TGF-β1 and decorin were consistently observed to have a similar cellular distribution, comparable to accumulations of both in hypertrophic scar tissue in humans.[62]

As demonstrated in this study, the PG composition of the developing lesion is dynamic and appears to be spatially related to the expression of PDGF and/or TGF-β1. Our challenge is to understand how this dynamic regulation of PGs affects lesion progression. Is the relative absence of perlecan in early lesions permissive to cellular infiltration and proliferation? Does early accumulation of versican in developing lesions enhance lipid deposition? Does the loss of versican from macrophage-rich areas, such as the plaque core and shoulders, affect plaque stability? Does biglycan association with elastin alter its polymerization or cross-linking and, therefore, the structural integrity of the vessel? Answers to questions such as these will help us elucidate the role of specific PGs in maintenance of the vessel wall integrity and the response of cells to locally released growth factors, such as PDGF and TGF-β1.

## Acknowledgments

The technical assistance of Roderick Browne and Jing Chen is gratefully acknowledged. We also thank the following colleagues for generously providing antibodies and reagents: Larry Fisher (Bone Research Branch, National Institute of Dental Research, NIH, Bethesda, MD) for the antibodies to decorin and biglycan; Richard LeBaron (Division of Life Sciences, University of Texas at San Antonio) for the versican-specific antibodies; Charles Underhill (Department of Anatomy, Georgetown University, School of Medicine, Washington, DC) for the biotinylated hyaluronan-binding protein; S. Koike (Mochida Pharmaceutical Co., Tokyo, Japan) for monoclonal antibody 007 to PDGF B-chain; and Glenn Pierce and John Tarpley (Amgen, Thousand Oaks, CA) for the monoclonal antibodies to PDGF A-chain.

## References

1. Wight TN: The vascular extracellular matrix. Atherosclerosis and Coronary Artery Disease. Edited by Fuster V, Ross R, Topol EJ. Philadelphia, Lippincott-Raven, 1996, pp 421–440
2. Nikkari S, Jarvelainen HT, Wight TN, Ferguson M, Clowes AW: Smooth muscle cell expression of extracellular matrix genes after arterial injury. Am J Pathol 1994, 144:1348–1356

3. Wolf Y, Rasmussen LM, Ruoslahti E: Antibodies against transforming growth factor-β1 suppress intimal hyperplasia in a rat model. J Clin Invest 1994, 93:1172–1178
4. Riessen R, Isner JM, Blessing E, Loushin C, Nikol S, Wight TN: Regional differences in the distribution of the proteoglycans biglycan and decorin in the extracellular matrix of atherosclerotic and restenotic human coronary arteries. Am J Pathol 1994, 144:962–974
5. Riessen R, Wight TN, Pastore C, Henley C, Isner JM: Distribution of hyaluronan during extracellular matrix remodeling in human restenotic arteries and balloon-injured rat carotid arteries. Circulation 1996, 93:1141–1147
6. Faggiotto A, Ross R, Harker L: Studies of hypercholesterolemia in the non human primate. I. Changes that lead to fatty streak formation. Arteriosclerosis 1984, 4:323–340
7. Yao L, Moody C, Schonherr E, Wight TN, Sandell LJ: Identification of the proteoglycan versican in aorta and SMCs by DNA sequence analysis, in situ hybridization, and immunohistochemistry. Matrix Biol 1994, 14:213–225
8. Brown DC, Vogel KG: Characteristics of the in vitro interaction of a small proteoglycan (PGII) of bovine tendon with type I collagen. Matrix 1989, 9:468–478
9. Schonherr E, Witsch-Prehm P, Harrach B, Robenek H, Rauterberg J, Kresse H: Interaction of biglycan with type I collagen. J Biol Chem 1995, 270:2776–2783
10. Baccarani-Contri M, Vincenzi D, Cicchetti F, Mori G, Pasquali-Ronchetti I: Immunocytochemical localization of proteoglycans within normal elastin fibers. Eur J Cell Biol 1990, 53:305–312
11. Schonherr E, Jarvelainen HT, Sandell LJ, Wight TN: Effects of platelet-derived growth factor and transforming growth factor-β1 on the synthesis of a large versican-like chondroitin sulfate proteoglycan by arterial smooth muscle cells. J Biol Chem 1991, 266:17640–17647
12. Schonherr E, Jarvelainen HT, Kinsella MG, Sandell LJ, Wight TN: Platelet-derived growth factor and transforming growth factor-β1 differentially affect the synthesis of biglycan and decorin by monkey arterial smooth muscle cells. Arterioscler Thromb 1993, 13:1026–1036
13. Papakonstantinou E, Karakiulakis G, Roth M, Block LH: Platelet derived growth factor stimulates the secretion of hyaluronic acid by proliferating human vascular smooth muscle cells. Proc Natl Acad Sci USA 1995, 92:9881–9885
14. Sambandam T, Baker JR, Christner JE, Ekborg SL: Specificity of low density lipoprotein-glycosaminoglycan interaction. Arterioscler Thromb 1991, 11:561–568
15. Cardoso L, Mourao PAS: Glycosaminoglycan fractions from human arteries presenting diverse susceptibilities to atherosclerosis have different binding affinities to plasma LDL. Arterioscler Thromb 1994, 14:115–124
16. Chen JK, Hoshi H, McKeehan WL: Transforming growth factor β specifically stimulates synthesis of proteoglycan in human adult arterial smooth muscle cells. Proc Nat Acad Sci USA 1987, 84:5287–5291
17. Levine JH, Moses HL, Gold LI, Nanney LB: Spatial and temporal patterns of immunoreactive transforming growth factor β1, β2, and β3 during excisional wound repair. Am J Pathol 1993, 143:368–380
18. Frank S, Madlener M, Werner S: Transforming growth factor β1, β2, and β3 and their receptors are differentially regulated during normal and impaired wound healing. J Biol Chem 1996, 271:10188–10193
19. Coker RK, Laurent GJ, Shahzeidi S, Lympany PA, du Bois RM, Jeffery PK, McAnulty RJ: Transforming growth factors-β1, -β2, and -β3 stimulate fibroblast procollagen production *in vitro* but are differentially expressed during bleomycin-induced lung fibrosis. Am J Pathol 1997, 150:981–991
20. Majesky M, Lindner V, Twardzik DR, Schwartz SM, Reidy MA: Production of transforming growth factor β1 during repair of arterial injury. J Clin Invest 1991, 88:904–910
21. Bahadori L, Milder J, Gold L, Botney M: Active macrophage-associated TGF-β co-localizes with type I procollagen gene expression in atherosclerotic human pulmonary arteries. Am J Pathol 1995, 146: 1140–1149
22. Ross R, Masuda J, Raines EW, Gown AM, Katsuda S, Sasahara M, Malden LT, Masuko, H, Sato, H: Localization of PDGF-B protein in macrophages in all phases of atherogenesis. Science 1990, 248: 1009–1012
23. Volker W, Schmidt A, Oortman W, Broszey T, Faber V, Buddecke E: Mapping of proteoglycans in atherosclerotic lesions. Eur Heart J 1990, 11(suppl E):29–40
24. Bianco P, Fisher LW, Young MF, Termine JD, Robey PG: Expression and localization of the two small proteoglycans biglycan and decorin in developing human skeletal and non-skeletal tissues. J Histochem Cytochem 1990, 38:1549–1563
25. du Cros D, LeBaron RG, Couchman JR: Association of versican with dermal matrices and its potential role in hair follicle development and cycling. J Invest Dermatol 1995, 105:426–431
26. Pierce G, Tarpley JE, Tseng J, Bready J, Chang D, Kenney WC, Ross R, Robson MC, Van de Berg J, Reid P, Kaufman S, Farrell CL: Detection of platelet-derived growth factor (PDGF)-AA in actively healing human wounds treated with recombinant PDGF-BB and absence of PDGF in chronic nonhealing wounds. J Clin Invest 1995, 96:1336–1350
27. Pelton R, Saxena B, Jones M, Moses HL, Gold LI: Immunohistochemical localization of TGFβ1, TGFβ2, and TGFβ3 in the mouse embryo: expression patterns suggest multiple roles during embryonic development. J Cell Biol 1991, 115:1091–1105
28. Underhill C, Nguyen H, Shizari M, Culty M: CD44 positive macrophages take up hyaluronan during lung development. Dev Biol 1993, 155:324–336
29. Gown, AM, Tsukada T, Ross R: Human atherosclerosis. II. Immunocytochemical analysis of the cellular composition of human atherosclerotic lesions. Am J Pathol 1986, 125:191–207
30. Masuda J, Ross R: Atherogenesis during low level hypercholesterolemia in the nonhuman primate. I. Fatty streak formation. Arteriosclerosis 1990, 10:164–177
31. Masuda J, Ross R: Atherogenesis during low level hypercholesterolemia in the nonhuman primate. II. Fatty streak conversion to fibrous plaque. Arteriosclerosis 1990, 10:178–187
32. Nunes I, Shapiro RL, Rifkin DB: Characterization of latent TGF-β activation by murine peritoneal macrophages. J Immunol 1995, 155: 1450–1459
33. Danielson KG, Baribault H, Holmes DF, Graham H, Kadler KE, Iozzo RV: Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility. J Cell Biol 1997, 136:729–743
34. Wainwright S, Biggs WD, Currey JD, Gosline JM: Mechanical Design in Organisms. Princeton, NJ, Princeton University Press, 1982, pp 124–127
35. Robbins R, Wagner WD, Register TC, Caterson B: Immunolocalization of proteoglycan types in aortas of pigeons with spontaneous or diet induced atherosclerosis. Am J Pathol 1989, 134:615–626
36. Lark M, Yeo T-K, Mar H, Lara S, Hellstrom I, Hellstrom K-E, Wight TN: Arterial chondroitin sulfate proteoglycan: localization with a monoclonal antibody. J Histochem Cytochem 1988, 36:1211–1221
37. Galis Z, Alaavi MZ, Moore S: In situ ultrastructural characterization of chondroitin sulfate proteoglycans in normal rabbit aorta. J Histochem Cytochem 1992, 40:251–263
38. Galis Z, Alaavi MZ, Moore S: Co-localization of aortic apolipoprotein B and chondroitin sulfate in an injury model of atherosclerosis. Am J Pathol 1993, 142:1432–1438
39. LeBaron R, Zimmerman DR, Ruoslahti E: Hyaluronate binding properties of versican. J Biol Chem 1992, 267:10003–10010
40. Wight TN, Lara S, Reissen R, LeBaron R, Isner J: Selective deposits of versican in the extracellular matrix of restenotic lesions from human peripheral arteries. Am J Pathol 1997, 151:963–973
41. Savani R, Wang C, Yang B, Zhang S, Kinsella MG, Wight TN, Stern R, Nance DM, Turley EA: Migration of bovine aortic endothelial cells after wounding injury: the role of hyaluronan and RHAMM. J Clin Invest 1995, 95:1158–1168
42. Brecht M, Mayer U, Schlosser E, Prehm P: Increased hyaluronate synthesis is required for fibroblast detachment and mitosis. Biochem J 1986, 239:445–450
43. Lin H, Ignatescu M, Wilson JE, Roberts CR, Horley KJ, Winters GL, Costanzo MR, McManus BM: Prominence of apolipoproteins B, (a), and E in the intimae of coronary arteries in transplanted human hearts: geographic relationship to vessel wall proteoglycans. J Heart Lung Transplant 1996, 15:1223–1232
44. Halpert I, Sires UI, Potter-Perigo S, Wight TN, Shapiro SD, Welgus HG, Wickline SA, Parks WC: Matrilysin is expressed by lipid laden macrophages at sites of potential rupture in atherosclerotic lesions and localizes to areas of versican deposition, a proteoglycan substrate for the enzyme. Proc Natl Acad Sci USA 1996, 93:9748–9753

45. Perides G, Asher RA, Lark MW, Lane WS, Robinson RA, Bignami A: Glial hyaluronate-binding protein: a product of metalloproteinase digestion of versican? Biochem J 1995, 312:377–384
46. Galis ZS, Sukhova GK, Lark MW, Libby P: Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest 1994, 94: 2493–2503
47. Lohmander LS: The release of aggrecan fragments into synovial fluid after joint injury and in osteoarthritis. J Rheumatol Suppl 1995, 43: 75–77
48. Lin H, Wilson JE, Roberts CR, Horley KJ, Winters GL, Costanzo MR, McManus BM: Biglycan, decorin, and versican protein expression patterns in coronary arteriopathy of human cardiac allografts: distinctness as compared to native atherosclerosis. J Heart Lung Transplant 1996, 15:1233–1247
49. Weiser M, Srieshaber NA, Schwartz PE, Majack RA: Perlecan regulates Oct-1 gene expression in vascular smooth muscle cells. Mol Biol Cell 1997, 8:999–1011
50. Van der Wal AC, Das PK, Tigges AJ, Becker AE: Macrophage differentiation in atherosclerosis: an *in situ* immunohistochemical analysis in humans. Am J Pathol 1992, 141:161–168
51. Kraiss LW, Raines EW, Wilcox JN, Seifert RA, Barrett TB, Kirkman TR, Hart CE, Bowen-Pope DP, Ross R, Clowes AW: Regional expression of the platelet-derived growth factor and its receptors in a primate graft model of vessel wall assembly. J Clin Invest 1993, 92:338–348
52. Alpers CE, Davis CL, Barr D, Marsh CL, Hudkins KL: Identification of platelet-derived growth factor A- and B-chains in human renal vascular rejection. Am J Pathol 1996, 148:439–451
53. Merwin JR, Newman W, Beall LD, Tucker A, Madri J: Vascular cells respond differentially to transforming growth factors-β1 and -β2 *in vitro*. Am J Pathol 1991, 138:37–51
54. Khalil N, Corne S, Whitman C, Yacyshyn H: Plasmin regulates the activation of cell-associated latent TGF-β1 secreted by rat alveolar macrophages after in vivo bleomycin-injury. Am J Respir Cell Mol Biol 1996, 15:252–259
55. Reilly KM, Melton DA: Short-range signaling by candidate morphogens of the TGFβ family and evidence for a relay mechanism of induction. Cell 1996, 86:743–754
56. Ruoslahti E, Yamaguchi Y: Proteoglycans as modulators of growth factor activities. Cell 1991, 64:867–869
57. DeLuca A, Manoranjan S, Baldi A, Giordano A, Iozzo, RV: Decorin-induced growth suppression is associated with up-regulation of p21, an inhibitor of cyclin-dependent kinases. J Biol Chem 1996, 271: 18961–18965
58. Aviezer D, Hecht D, Safran M, Eisinger M, David G, Yayon A: Perlecan, basal lamina proteoglycan, promotes basic fibroblast growth-factor receptor binding, mitogenesis, and angiogenesis. Cell 1994, 79:1005–1013
59. Raines E, Ross R: Compartmentalization of PDGF on extracellular binding sites dependent on exon-6-encoded sequences. J Cell Biol 1992, 116:533–543
60. Hildebrand A, Romaris M, Rasmussen LM, Heinegard D, Twardzik DR, Border W, Ruoslahti E: Interaction of the small interstitial proteoglycans biglycan, decorin, and fibromodulin with transforming growth factor β. Biochem J 1994, 302:527–534
61. Border WA, Noble NA, Yamamoto T, Harper J, Yamaguchi Y, Pierschbacher MD, Ruoslahti E: Natural inhibitor of transforming growth factor-β protects against scarring in experimental kidney disease. Nature 1992, 360:361–364
62. Scott P, Dodd CM, Tredget EE, Ghahary A, Rahemtulla F: Immunohistochemical localization of the proteoglycans decorin, biglycan, and versican and transforming growth factor-β in human post-burn hypertrophic and mature scars. Histopathology 1995, 26:423–431